

# Brush cytology in the assessment of pancreatico–biliary strictures: a review of 406 cases

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## Abstract

**Aims**—To assess the accuracy of brush cytology in patients investigated for pancreatico–biliary strictures.

**Methods**—All pancreatico–biliary brush cytology specimens submitted from two major teaching hospitals over a 6.5 year period were reviewed. Four hundred and forty eight satisfactory specimens from 406 patients with adequate clinical and/or pathological follow up data were examined in the study period.

**Results**—Two hundred and forty six patients (60.6%) were shown to have neoplastic strictures. One hundred and forty seven tumours were identified cytologically, including 87 of 146 pancreatic carcinomas, 29 of 47 cholangiocarcinomas, one of one bile duct adenoma, four of seven carcinomas of the gallbladder, eight of 13 ampullary carcinomas, two of three ampullary adenomas, 10 of 16 malignancies of undetermined origin, none of two islet cell tumours, one of three hepatocellular carcinomas, and five of eight metastatic tumours. The three adenomas identified on brush cytology could not be distinguished from adenocarcinoma morphologically. One hundred and sixty patients (39.4%) had benign strictures, most often as a result of chronic pancreatitis and bile duct stones. There were three false positive cytological diagnoses mainly as a result of the misinterpretation of cases with relatively scant and/or degenerative atypical epithelial cells. Forty one cases were reported as atypical or suspicious of malignancy on brush cytology, of which 29 were ultimately shown to have carcinoma. The overall diagnostic sensitivity and specificity were 59.8% and 98.1%, respectively. The sensitivity increased from 44.3% in the initial third of cases to 70.7% in the final third of cases examined in the series.

**Conclusions**—Brush cytology, in conjunction with other clinical and radiological investigations, is a useful technique in the assessment of patients with suspected pancreatico–biliary neoplasia.

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**Keywords:** brush cytology; pancreatico–biliary strictures; pancreatico–biliary neoplasia

Biliary and pancreatic duct strictures are most often caused by inflammatory or neoplastic disorders involving the pancreas, biliary tree,

gallbladder, or ampulla. Conservative management with endoscopic stenting is used in most benign strictures, and also in many patients with malignant tumours, most of whom have locally advanced disease or distant metastases at presentation, precluding the possibility of curative resection.<sup>1–3</sup> In such cases, an accurate tissue diagnosis, preferably obtained without the need for laparotomy, helps to plan further management, particularly if patients with neoplasia are to be included in therapeutic clinical trials. Conversely, surgical intervention might be appropriate in some patients with apparently localised disease, regardless of the cause of the stricture, and also for symptomatic relief in patients with irresectable tumours and complications caused by local spread. Although preoperative histological or cytological confirmation of neoplasia is less crucial in these cases, ideally a tissue diagnosis should be obtained whenever possible.<sup>1–3</sup>

Biliary and pancreatic duct lesions are not always readily accessible to biopsy, and cytological techniques have become the initial diagnostic modality in many cases. In general, the examination of bile, and of pancreatic or duodenal fluid aspirates, has been disappointing because cellular preservation is often poor, relatively few malignancies are identified, and occasional false positive results have been described.<sup>4–5</sup> Percutaneous radiologically guided fine needle aspiration (FNA) is a very accurate technique, particularly in the diagnosis of pancreatic cancer, but is operator dependent and requires a sufficiently distinct mass lesion for targeting. Concerns have also been raised regarding potential tumour spread after percutaneous pancreatic biopsy,<sup>6</sup> although data are conflicting.<sup>7–8</sup> Brush cytology performed at endoscopic retrograde cholangio-pancreatography (ERCP) has now become the preferred initial method of pursuing tissue diagnosis in many patients with pancreatico–biliary strictures. The technique has a low complication rate and allows sampling from most sites within the pancreatic and biliary duct systems. Brushing specimens usually yield well preserved, cellular samples suitable for cytological analysis as indicated by the low unsatisfactory rate (around 5%) in most studies.<sup>4–5</sup>

The diagnostic specificity of biliary brush cytology is very high and few false positive diagnoses have been reported. The major limitation of the technique has been the relatively modest diagnostic sensitivity recorded in most studies to date. Kurzwinski and colleagues<sup>4</sup>

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Table 1 Correlation between cytological diagnosis and the final clinicopathological diagnosis for the 406 patients

Clinicopathological diagnosis	Cytological diagnosis		
	Negative	Atypical	Malignant
Benign (n = 160)	145	12	3
Neoplastic (n = 246)			
Carcinoma of pancreas (n = 146)	40	19	87
Cholangiocarcinoma (n = 48)	12	6	30
Ampullary neoplasm (n = 16)	6	0	10
Gallbladder carcinoma (n = 7)	2	1	4
Malignant, site ND (n = 16)	3	3	10
Islet cell tumour (n = 2)	2	0	0
HCC (n = 3)	2	0	1
Metastasis (n = 8)	3	0	5
Total	215	41	150

The ampullary neoplasm category included three adenomas of ampulla and the cholangiocarcinoma group included one bile duct adenoma. HCC, hepatocellular carcinoma; ND, not determined.

reviewed six early series, comprising between nine and 65 patients, and found the mean sensitivity to be 59% (range 54–66%). Similarly, Foutch<sup>5</sup> documented a mean sensitivity of 59% in an analysis of 10 studies, comprising between 19 and 72 patients, although the range was wider (42–85%) in these reports. More recently, five large studies of brush cytology have recorded sensitivities of only 35–48% in patients with pancreatico–biliary malignancy.<sup>9–13</sup> However, not all the data are comparable. Some studies have examined only pancreatic duct<sup>14–16</sup> or bile duct strictures,<sup>11–17</sup> although most have included a variable proportion of ampullary, biliary, and pancreatic duct lesions. Equally, there is inconsistency in the range and assessment of cytological diagnoses. Although some reports describe only definite positive or negative findings, many authors have included an indeterminate diagnostic category variably classified as atypical, dysplastic, or suspicious of malignancy.<sup>9–10 18–20</sup> In addition, although Desa and colleagues<sup>17</sup> and Bardales and colleagues<sup>21</sup> included suspicious cases with definite malignancies in the analysis of their data, most other authors have regarded equivocal reports as negative in the assessment of diagnostic sensitivity.

To assess further the accuracy of brush cytology in pancreatico–biliary strictures, and to assess the outcome in patients with atypical or equivocal cytological changes, we have reviewed our experience over the 6.5 year period January 1993 to June 1999.

### Methods

All pancreatico–biliary brush cytology specimens submitted from the Western Infirmary, Glasgow and the Royal Infirmary, Glasgow between January 1993 and June 1999 were reviewed. Both hospitals have a major referral interest in the management of pancreatico–biliary disease and clinicians followed up their patients regularly. Four hundred and eighty nine consecutive specimens were received from 440 patients in the review period. For inclusion in the study, patients had to have a definite final benign or malignant diagnosis based either on independent histological or cytological sampling, or on clinical and radiological follow up data. The latter were obtained by case record review and by correspondence with referring

clinicians and general practitioners. A minimum six months healthy clinical follow up period was required for confirmation of a negative cytological diagnosis.

Fifteen specimens from 15 patients (3.4%) were excluded from the study because adequate follow up data were not available (six cases), or because the patients died of uncertain or unrelated causes within six months of investigation (nine cases). Twenty specimens from 19 patients (4.3%) were considered insufficiently cellular for cytological diagnosis. Six of these patients were eventually shown to have malignant disease, nine had benign strictures, whereas the remaining four patients were lost to follow up. These unsatisfactory specimens were excluded from further assessment. Six further patients had initial unsatisfactory specimens but subsequent sampling proved adequate for diagnosis. No significant change in the unsatisfactory rate occurred throughout the study period and there was no clear correlation between unsatisfactory sampling and the suspected tumour site. Thirty nine patients had two satisfactory specimens and one patient underwent sampling on four occasions. The study group therefore comprised 448 specimens obtained from 406 patients. There were 183 male and 223 female patients, with an age range 33–96 years (mean, 67.4). The final diagnosis was confirmed by histological and/or independent cytological assessment in 171 patients, and by clinical and radiological follow up data in 235 patients.

The specimens were obtained at ERCP by passing the brush over a guide wire placed across the stricture. The brushes were placed in normal saline or sent directly to the laboratory. Routinely, six cytopsin preparations were prepared and stained according to Papanicolaou's method. Further preparations (four to six cytopsin) were examined at the discretion of the cytopathologist in problematic cases. Most specimens were reported as definitely benign or malignant but in some an equivocal diagnosis, variably described as atypical, dysplastic, or suspicious/suggestive of malignancy, was made. Such equivocal diagnostic cases were grouped as atypical in our study.

In those patients who had two or more brush cytology specimens the more/most positive cytological diagnosis was used for clinicopathological correlation.

Diagnostic sensitivity was calculated as true positive (TP)/TP + false negative (FN) results. Specificity was true negative (TN)/TN + false positive (FP) results. Positive predictive value (PPV) was TP/TP + FP, and negative predictive value (NPV) was TN/TN + FN results. For the purposes of analysis atypical reports were considered to be negative.

### Results

Table 1 summarises the correlation between the cytological diagnoses and the final clinicopathological diagnoses for the 406 patients.

Two hundred and fifteen patients (53%) had a negative cytological diagnosis. These specimens included clusters or regular flat sheets of uniform ductal epithelium. The cellularity

ranged from scant (one to two groups/cytospin preparation) to highly cellular. The nuclei were round to oval with only slight variation (less than  $\times 2$ ) in size. Inflammatory cells and crystalline material were seen in some cases. Cells reminiscent of squamous metaplasia with moderately enlarged nuclei, dense cytoplasm, and a distinct cytoplasmic margin were variably present. Such reactive changes were considered to be within the spectrum of negative cytology. Subsequent pathological or clinical data confirmed a benign outcome in 145 patients but 70 were eventually shown to have neoplastic disease. Of the false negative cases; 40 patients had adenocarcinoma of the pancreas; 12 cholangiocarcinoma; six ampullary neoplasms (five adenocarcinomas and one adenoma); two patients each had carcinoma of the gallbladder, hepatocellular carcinoma, and islet cell tumour of the pancreas; and three patients were thought to have a biliary stricture secondary to metastatic carcinoma. Three patients with false negative cytology had presumed pancreatico–biliary malignancy but the exact anatomical site was not determined.

One hundred and fifty patients (36.9%) had a cytological diagnosis of malignancy. The specimens were usually of moderate to high cellularity, although benign elements were invariably present and usually accounted for most of the cell content. Malignant elements typically comprised loosely cohesive, crowded, and disorganised cell groups, together with small acinar clusters and single pleomorphic cells. Nuclei were enlarged and irregular, and

nucleoli were often evident. Necrosis was an occasional feature. In 148 cases, the malignant specimens were reported as consistent with an adenocarcinoma of pancreatico–biliary origin. The cytological appearances were considered more suggestive of hepatocellular carcinoma in one case, a diagnosis supported by *in situ* hybridisation for albumin messenger RNA,<sup>22</sup> and in a second patient with known gastric cancer the brush cytology was considered consistent with metastatic carcinoma.

Clinicopathological correlation confirmed the malignant diagnoses in 144 patients. Eighty seven patients had pancreatic adenocarcinoma (including one mixed adenocarcinoma/small cell neuroendocrine carcinoma), 29 cholangiocarcinoma, eight periampullary adenocarcinoma, four adenocarcinoma of the gall bladder, one hepatocellular carcinoma, and five metastatic malignancy. Four of the latter cases were considered consistent with bile duct origin on brush cytology. However, subsequent resection in one patient revealed a biliary stricture secondary to metastatic lobular carcinoma of breast rather than cholangiocarcinoma (fig 1). The history of breast cancer was not available at the time of cytological diagnosis. Three further patients were clinically suspected to have biliary compression as a result of metastatic colonic cancer, gastric carcinoma, and malignant melanoma, respectively, but none had histological confirmation. In 10 patients with positive cytology and clinically confirmed malignant disease, the precise site of tumour origin was not determined, although all were thought to have primary pancreatico–biliary carcinoma.

Three patients with positive cytology were found to have *in situ* neoplasia (adenomas) involving the ampulla or the distal common bile duct on pancreatico–duodenectomy (two cases) or biopsy (one case) without evidence of invasive malignancy. On cytological review, these cases showed a general preservation of architectural arrangement, with relatively scant dissociated malignant cells (fig 2). However, cell clusters similar to those seen in confirmed invasive adenocarcinoma were present in two of these cases. Conversely, confirmed invasive adenocarcinomas in our series not uncommonly exhibited an identical cytological pattern to those of the adenomas. These cases were regarded as true positive diagnoses in the series.

There were three non-neoplastic, false positive diagnoses. The first involved a 42 year old woman with a history of primary sclerosing cholangitis who developed a proximal common bile duct stricture. The stricture was resected after the cytological diagnosis of carcinoma but histological examination revealed only reactive and inflammatory biliary epithelial changes associated with biliary and intrahepatic microcalculi. A second brush sample taken six months postoperatively was considered highly atypical, but without definite evidence of malignancy. The patient was alive without evidence of tumour 24 months postoperatively. Review of the cytological preparations from the

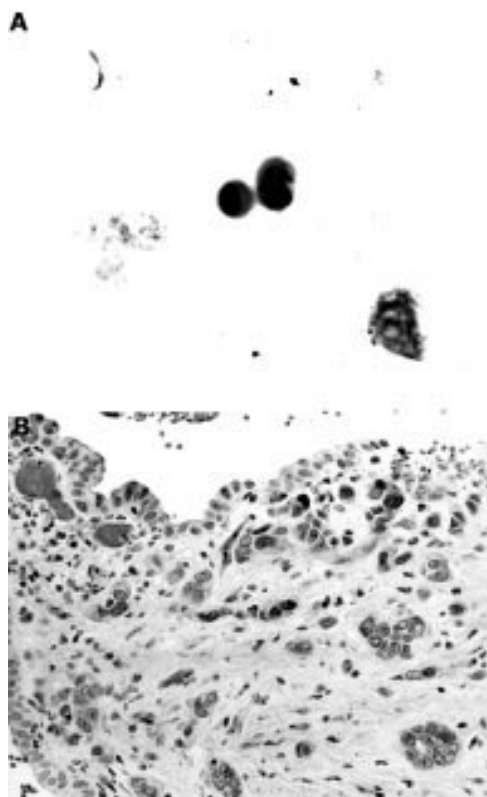
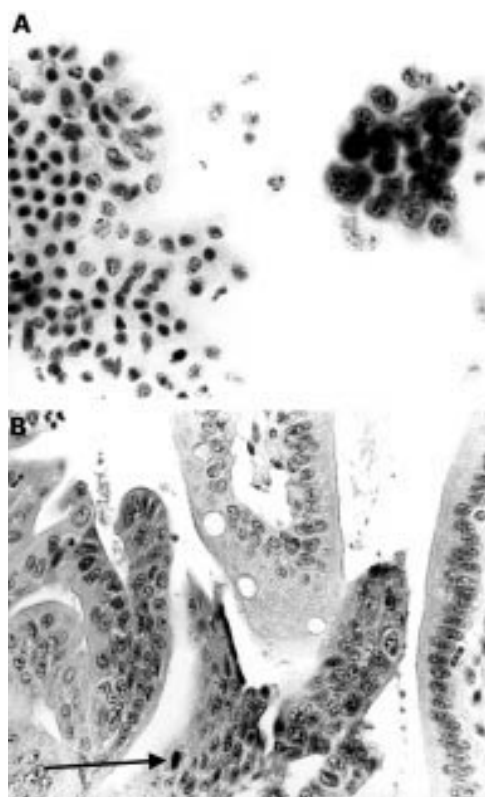


Figure 1 Metastatic lobular carcinoma of breast. (A) Brush cytology showing a small number of malignant cells with eccentric nuclei. (B) Resection showing infiltrating lobular carcinoma adjacent to surface epithelium (above).



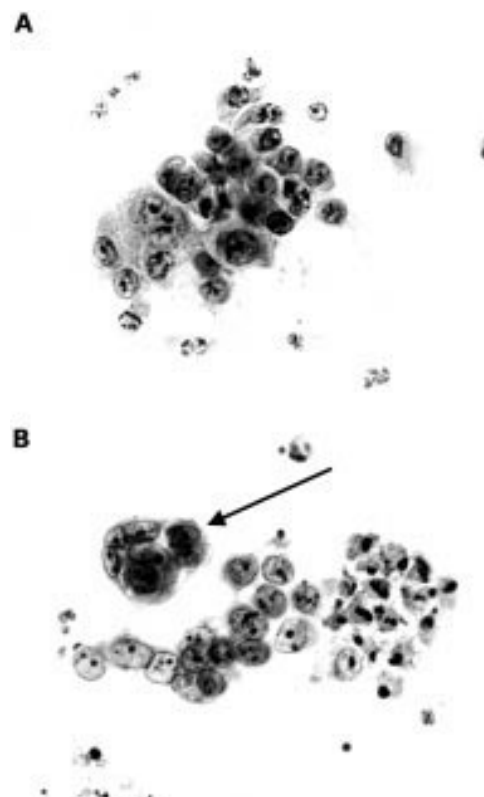


**Figure 2** Bile duct adenoma. (A) Brush cytology showing a loosely cohesive and crowded epithelial group exhibiting malignant nuclear features. A group of normal ductal cells is present for comparison (left). (B) Resection showing papillary adenoma with focal high grade dysplasia. Note the mitotic figure (arrow).

initial sample revealed three dimensional clusters of crowded gland-like groups with peripherally distributed and moulded nuclei showing a coarse chromatin pattern and distinct nucleoli (fig 3). Scattered single cells showing similar pronounced nuclear atypia were also present and there was focal cellular debris interpreted as necrosis. There were also very pronounced degenerative changes affecting both atypical and otherwise normal ductal cells.

The second false positive case was a 63 year old woman with a suspected carcinoma of the head of the pancreas. Brush cytology showed a small number of highly atypical but variably degenerate epithelial groups that were reported as consistent with an adenocarcinoma. However, no tumour was evident at laparotomy. The patient died of postoperative mesenteric ischaemia. No tumour was identified at necropsy, although histological sampling of the pancreas and biliary system was limited, and slides were not available for review.

The final false positive case involved a woman of 67 years with a periampullary stricture. Two brush cytology samples were taken, the first being considered atypical and the second reported as showing a small number of epithelial groups consistent with adenocarcinoma. The patient was not considered a suitable candidate for surgery in view of her poor general health and was therefore managed conservatively. Clinical follow up and repeat ERCP 11 months after presentation showed



**Figure 3** False positive brush cytology (case 1). (A) Loosely cohesive epithelial group showing nuclear variation, coarse chromatin pattern and prominent nucleolation. (B) Moulded atypical nuclei with acinar type cluster (arrow). Note the degenerative cellular changes (right).

common bile duct stones but the stricture had resolved and there was no evidence of neoplasia. Review of the second cytology specimen revealed a small number of highly atypical but focally degenerative epithelial cells insufficient for a diagnosis of carcinoma.

Two of the false positive specimens were reported within the initial third of cases examined in this series, whereas the last was a more recent case. In retrospect, the degenerative changes evident in all cases, and the relatively scant abnormal cells in two specimens, should have led to an equivocal (atypical) rather than definite malignant diagnosis.

Brush cytology specimens from 41 patients (10.1%) were categorised as atypical. These cases exhibited a wide spectrum of cytological appearances that had been variably reported as atypical, dysplastic, or suspicious/suggestive of malignancy. Some showed a predominantly benign pattern, but with focal architectural disturbance or unusually pronounced reactive nuclear changes. At the other extreme were specimens in which cells consistent with carcinoma were present but considered insufficiently numerous or well preserved for a definite malignant diagnosis. In some cases, the presence of pronounced inflammation, evidence of calculus disease, or history of recent surgical manipulation including stenting led to an equivocal cytodiagnosis. Clinicopathological follow up data revealed that 29 patients with atypical cytological findings proved to have malignant disease whereas 12 had a benign

outcome. The most common causes of benign atypical cytology were chronic pancreatitis and calculus disease. The proportion of atypical cases was relatively constant throughout our study period, although slightly fewer atypical cases were reported during the earliest time period.

Of the 40 patients with two or more brush specimens, 22 had a benign outcome and 18 were eventually found to have malignant disease. Ten of the patients with carcinoma had positive cytology only on a subsequent specimen, and two further patients had two atypical diagnoses; five patients with carcinoma had two negative brush samples and one patient had negative and atypical diagnoses. Of the 22 patients with benign disease, 12 had two negative cytology reports and 10 had at least one atypical diagnosis. Two of the latter patients also had false positive malignant diagnoses as described above. Overall, the later brush specimen provided a more accurate diagnosis in 16 cases, was identical to the first sample in 20 cases, and was less accurate in four cases.

On the basis of clinical, radiological, and pathological findings, of the 246 patients with established neoplastic disease 146 were considered to have pancreatic carcinoma, 48 primary bile duct neoplasia (47 cholangiocarcinomas and one adenoma), 16 primary ampullary tumours (13 adenocarcinomas and three adenomas), seven carcinoma of the gallbladder, two islet cell tumours, three hepatocellular carcinoma, and eight metastatic carcinoma. In 16 patients the precise tumour origin was not determined. There was no significant difference in the proportion of pancreatic, bile duct, ampullary, or gall bladder neoplasms identified by brush cytology in our study (87 of 146 (59.6%), 30 of 48 (62.5%), 10 of 16 (62.5%), and four of seven (57%), respectively).

One hundred and sixty patients had benign disease, most commonly biliary stones (51 cases) and chronic pancreatitis (40 cases). Twenty one patients had a variety of benign diagnoses including primary sclerosing cholangitis, duodenal diverticulum, primary parenchymal liver disease, intra-abdominal sepsis, sphincter hypertension, and benign pancreatic neoplasm (serous cystadenoma). The remaining patients with a benign outcome had no definite diagnosis, although stones and pancreatitis were suspected in many cases.

Overall, therefore, there were 147 TP diagnoses, 157 TN diagnoses, 99 FN diagnoses, and three FP diagnoses. The diagnostic sensitivity, specificity, PPV, and NPV of brush cytology for the series were 59.8%, 98.1%, 98%, and 61.3%, respectively. Most of these parameters improved over the course of the study period as summarised in table 2. For the

last 136 patients (approximately one third of the total group) the diagnostic sensitivity was 70.7%.

## Discussion

The examination of brush cytology specimens has become an established diagnostic technique in the investigation of patients with suspected pancreatic, bile duct, gallbladder, and ampullary tumours. We have reviewed 448 consecutive brush samples obtained from 406 patients and correlated the findings with pathological and clinical outcomes. To our knowledge, this is the largest series of pancreatico–biliary brush cytology specimens yet reported.

As with previous studies, we found that the brush cytology technique produced cellular samples of good quality in most instances. Only 26 of 489 (5.3%) specimens were considered inadequate for diagnosis. Seven patients with initial unsatisfactory cytology samples underwent repeat endoscopy and an adequate specimen was obtained in six cases. The number of unsatisfactory specimens has not been specifically noted in many previous studies although rates of 0–6% were documented in three series.<sup>9 11 18</sup> There was no clear correlation between unsatisfactory sampling and the site of stricture in our patients, and the proportion of cases with insufficient material was fairly constant throughout our study period.

We found that brush cytology accurately identified 147 of 246 (59.8 %) neoplasms in our series, a similar result to those of most early, smaller studies reviewed by Kurzwinski and colleagues<sup>4</sup> and by Foutch.<sup>5</sup> Other more recent series have reported somewhat lower diagnostic sensitivities. Kocjan and Smith<sup>9</sup> analysed biliary duct brushings from 131 patients in whom a histological correlation was available. The diagnostic sensitivity for patients with biopsy confirmed carcinoma was 44%, excluding dysplastic cytological diagnoses. Lee and colleagues<sup>10</sup> examined brush cytology from 168 pancreatico–biliary strictures in 149 patients and found the diagnostic sensitivity to be 37%. Ponchon *et al* identified 45 of 127 (35%) primary bile duct carcinomas using brush cytology.<sup>11</sup> Sturm and colleagues<sup>12</sup> reviewed brush cytology from 294 patients with biliary strictures, including 220 patients with malignancy; 79 carcinomas (36%) were identified cytologically. Recently, Logrono *et al* reported 48% sensitivity for malignancy in an analysis of 183 pancreatico–biliary brush cytology specimens.<sup>13</sup>

There are several possible explanations for the limited sensitivity of brush cytology in assessing pancreatic and biliary carcinomas but these can be broadly separated into sampling and interpretative errors. The former might occur when tumours at these sites show a predominantly submucosal spread, with limited or absent surface epithelial abnormality.<sup>4 5 9</sup> Similarly, strictures might be caused by external compression—for example, by lymph node metastasis—without directly involving the ductal epithelium. The site of the tumour might also be important. Several studies have shown

Table 2 Changes in the accuracy of brush cytology during the study period

Patients	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1–135	35/79 (44.3)	54/56 (96.4)	35/37 (94.6)	54/98 (55.1)
136–270	47/75 (62.7)	60/60 (100)	47/47 (100)	60/88 (68.2)
271–406	65/92 (70.7)	43/44 (97.7)	65/66 (98.5)	43/70 (61.4)
Overall	147/246 (59.8)	157/160 (98.1)	147/150 (98)	157/256 (61.3)

NPV, negative predictive value; PPV, positive predictive value.

that diagnostic accuracy is greatest for ampullary neoplasms, intermediate for cholangiocarcinoma, and lowest for pancreatic carcinoma, particularly for tumours in the pancreatic tail.<sup>4 5 14 16 23</sup> However, we were unable to confirm this finding in our series. Interpretative errors are more likely to occur in well differentiated carcinomas, in which the cytological abnormality may be minimal,<sup>18</sup> or in specific tumour subtypes, such as papillary or mucinous carcinomas, which might not be recognised by pathologists.<sup>9</sup> Kocjan and Smith re-examined cytological preparations from 20 confirmed false negative cases and, on review, considered eight to show features of carcinoma or dysplasia.<sup>9</sup> A similar review by Logrono and colleagues<sup>13</sup> found that interpretative and technical errors accounted for 12 of 36 false negative cytodiagnoses, the remainder being the result of sampling error. These studies suggest that a considerable number of false negative errors are related to cytological undergrading and it is possible, therefore, that ancillary techniques based on tumour biology, such as the identification of p53 immunoreactivity or K-ras mutations, may enhance diagnostic sensitivity in morphologically negative or equivocal cases.<sup>12 15 24 25</sup>

It is noteworthy that the diagnostic accuracy of brush cytology increased during the course of our study. In particular, the sensitivity improved from 44.3% in the initial third to 70.7% in the final third of cases. We suspect that this improvement was mainly caused by the greater experience of pathologists in interpreting pancreatico-biliary cytology specimens, but it may also have reflected better clinical sampling of malignant strictures. Our data also supported the use of repeat sampling in patients with suspected malignancy in whom initial cytology had proved negative because 10 of 18 patients with carcinoma had positive cytology only on a second brush specimen. Overall, subsequent examination proved more accurate in 16 of 40 patients undergoing two procedures, similar in 20 cases, and less accurate in only four samples. Rabinovitz *et al* also showed an improved diagnostic sensitivity with repeat sampling in 65 patients with biliary strictures.<sup>26</sup> The diagnostic sensitivity increased from 40% with one brush sample to 62% after the examination of multiple specimens. In their study, three negative specimens excluded a diagnosis of malignancy.

Previous studies have shown that a diagnosis of carcinoma on brush cytology is highly reliable and many have reported 100% diagnostic specificity. There were three false positive diagnoses in our study (specificity 98.1%), principally as a result of the overinterpretation of atypical epithelial changes in patients with sclerosing cholangitis, bile duct stones, and pancreatitis. On review, it was felt that the cytological appearances in each case should have been regarded as equivocal because of the degenerative changes in all specimens and the relatively scant atypical epithelial cells in two. False positive pancreatico-biliary brush cytology diagnoses have previously occurred as a result of the

misinterpretation of low grade dysplasia, reactive papillary changes with epithelial atypia, intestinal metaplasia of biliary epithelium, and the effects of previous bile duct stenting.<sup>13 18 20 27</sup> Sturm *et al* also reported two false positive cytological diagnoses among 74 patients with benign biliary strictures.<sup>12</sup> Both patients were thought to have postsurgical bile duct stenoses and, interestingly, K-ras mutations were also identified in each case. However, neither patient had evidence of malignancy on clinical follow up. A further example of cytological overdiagnosis was described by Desa *et al*,<sup>17</sup> who recorded a case of pancreatic duct hyperplasia in which cytology had been reported as highly suspicious of carcinoma. Thus, it would appear from some recent large series that atypical but reactive epithelial changes may closely mimic malignancy in occasional pancreatico-biliary brush cytology specimens.

Three patients in our series with positive cytology proved to have adenomas involving the ampulla or distal common bile duct, but no evidence of invasive malignancy on biopsy and clinical follow up (one case) or on pancreaticoduodenectomy (two cases). Bardales and colleagues<sup>21</sup> reported three similar cases in which duodenal or biliary brush cytology from periampullary villous adenomas was considered consistent with carcinoma cytologically. In addition, Sawada *et al* recorded a patient with positive pancreatic duct cytology in whom resection revealed extensive "intraepithelial carcinoma" but no invasive malignancy.<sup>14</sup> Although such cases arguably represent false positive cytological diagnoses, we do not feel that such an interpretation is justified. As with similar neoplasms in the gastrointestinal tract, adenomas involving the biliary system are considered to have malignant potential, and the operative procedures were considered appropriate in both patients undergoing resection in our series. We feel that these cases illustrate the inability of cytological assessment to distinguish reliably between in situ and invasive neoplasia in the pancreatico-biliary system, as in other sites. This is not surprising considering that histological examination is necessary to demonstrate invasion of stroma by tumour. Although the cytomorphology of the adenomatous cases we examined was characterised by a relative preservation of architectural pattern, identical appearances were seen in other specimens with confirmed invasive carcinoma. We previously described similar findings in a patient with an intraductal papillary mucinous tumour of the pancreas in which only microscopic invasive foci were seen.<sup>28</sup> Although most patients with positive pancreatico-biliary cytology will prove to have invasive carcinoma, like Bardales and colleagues,<sup>21</sup> we feel that it is impossible to distinguish reliably between in situ and invasive neoplasia in cytological specimens.

In our study, 41 pancreatico-biliary brush cytology specimens (10.1%) resulted in an equivocal (atypical) cytology report. There was no reduction in the proportion of atypical cases over the course of the study and, indeed, the



lowest percentage was reported in the initial period. Thus, an indeterminate cytological assessment was not simply an effect of inexperience. Whereas some studies have recorded only definite positive or negative cytological findings, many authors have included an indefinite category, although the terminology has varied. Layfield and colleagues<sup>19</sup> provided cytological criteria for low and high grade dysplasia in addition to normal, reactive, and malignant cellular appearances. The authors recorded dysplasia in 19 of 108 (18%) pancreatic and biliary duct brush specimens. Ten of 12 patients with high grade dysplasia and three of seven with low grade dysplasia proved to have malignancy, whereas two additional patients had histologically confirmed dysplasia but no evidence of invasive carcinoma. In a subsequent series,<sup>10</sup> the authors recorded that 20 of 32 patients with cytological dysplasia had clinicopathological evidence of malignancy. Using the same cytological criteria, Kocjan and Smith<sup>9</sup> reported dysplasia in 10 of 131 (7.6%) bile duct brush samples; seven patients had histological evidence of malignancy whereas three had a benign outcome. Ryan and Baldauf<sup>20</sup> described atypical cytological findings in 22 of 38 brush specimens from patients with pancreatobiliary malignancies but also in two of 13 with benign disease. In our retrospective study, the atypical cases clearly represented a heterogeneous group, and some would have been more accurately classified as low or high grade dysplasia using the criteria of Layfield *et al.*<sup>19</sup> However, in common with previous studies, we found that approximately two thirds of patients with atypical cytological diagnoses were ultimately shown to have carcinoma. Therefore, although an atypical or dysplastic cytological diagnosis merits further investigation it could not be considered sufficiently specific, in isolation, to support a radical therapeutic procedure.

In summary, brush cytology identified 147 of 246 (59.8%) neoplasms in a series of 406 consecutive patients evaluated over a 6.5 year period in our institution. Three false positive cytological diagnoses occurred as a result of misinterpretation of atypical but degenerative epithelial changes. The diagnostic sensitivity improved during the study period. Although the limitations of the technique must be recognised, brush cytology is useful in the initial investigation of patients with suspected pancreatobiliary neoplasia.

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